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The designation on the cover of this publication, "USP NF 2007," is for ease of identification only. The publication contains two separate compendia: *The United States Pharmacopeia*, Thirtieth Revision, and the *National Formulary*, Twenty-Fifth Edition.

THE UNITED STATES PHARMACOPEIAL CONVENTION
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SIX-MONTH IMPLEMENTATION GUIDELINE

Beginning with *USP30–NF25*, the *United States Pharmacopeia–National Formulary* and its *Supplements* will become official **six months** after being released to the public. The *USP–NF*, which is released on November 1 of each year, will become official on May 1 of the following year.

This change was adopted to give users more time to bring their methods and procedures into compliance with new and revised *USP–NF* requirements.

The table below describes the new official dates. The 2006 *USP29–NF24*, and the *Supplements* and *Interim Revision Announcements (IRAs)* to that edition, will be official until May 1, 2007, at which time the *USP30–NF25* becomes official.

Publication	Release Date	Official Date	Official Until
USP30–NF25	Nov. 1, 2006	May 1, 2007	May 1, 2008 (except as superceded by *Supplements, IRAs,* and *Revision Bulletins*)
First Supplement	Feb. 1, 2007	Aug. 1, 2007	May 1, 2008 (except as superceded by *Second Supplement, IRAs,* and *Revision Bulletins*)
Second Supplement	June 1, 2007	Dec. 1, 2007	May 1, 2008 (except as superceded by *IRAs* and *Revision Bulletins*)
USP31–NF26	Nov. 1, 2007	May 1, 2008	May 1, 2009 (except as superceded by *Supplements, IRAs,* and *Revision Bulletins*)

IRAs will continue to become official on the first day of the second month of the *Pharmacopeial Forum (PF)* issue in which they are published as final. For instance, *IRAs* published as final in the May-June *PF* (issue 3) will become official on June 1. This table gives the details of the *IRAs* that will apply to *USP29–NF24* and *USP30–NF25.*

*IRA**	Release Date	Official Date	Revises
Jan. 1, 2007 *IRA, PF* 33(1)	Jan. 1, 2007	Feb. 1, 2007	*USP29–NF24* and its *Supplements*
Mar. 1, 2007 *IRA, PF* 33(2)	Mar. 1, 2007	April 1, 2007	*USP29–NF24* and its *Supplements*
May 1, 2007 *IRA, PF* 33(3)	May 1, 2007	June 1, 2007	*USP30–NF25*
July 1, 2007 *IRA, PF* 33(4)	July 1, 2007	Aug. 1, 2007	*USP30–NF25* and *First Supplement*
Sept. 1, 2007 *IRA, PF* 33(5)	Sept. 1, 2007	Oct. 1, 2007	*USP30–NF25* and *First Supplement*
Nov. 1, 2007 *IRA, PF* 33(6)	Nov. 1, 2007	Dec. 1, 2007	*USP30–NF25* and its *Supplements*
Jan. 1, 2008 *IRA, PF* 34(1)	Jan. 1, 2008	Feb. 1, 2008	*USP30–NF25* and its *Supplements*
Mar. 1, 2008 *IRA, PF* 34(2)	Mar. 1, 2008	April 1, 2008	*USP30–NF25* and its *Supplements*

*NOTE—Beginning January 1, 2007, USP will cease identifying *IRAs* numerically (*First, Second,* etc.) and instead will designate them by the date on which they are published.

Revision Bulletins published on the USP website will continue to become official immediately upon publication, unless the *Revision Bulletin* specifies otherwise.

General Chapters, monographs, or monograph revisions that contain a specific official date shall continue to become official upon such specified date, which supercedes the general official date for the publication.

For more information about the change in official dates, please visit the USP website at http://www.usp.org.

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Extraction Solvent C) or in an oven at 100°, dry at 105° for 1 hour, cool in a desiccator, and weigh. Calculate the total extractables, in mg, by the formula:

$$2(W_U - W_B)$$

in which W_U is the weight, in mg, of residue found in the sample extract aliquot; and W_B is the weight, in mg, of residue found in the blank solution aliquot.

⟨391⟩ EPINEPHRINE ASSAY

USP Reference Standards ⟨11⟩—*USP Epinephrine Bitartrate RS.*

Ferro-citrate Solution—On the day needed, dissolve 1.5 g of ferrous sulfate in 200 mL of water to which have been added 1.0 mL of dilute hydrochloric acid (1 in 12) and 1.0 g of sodium bisulfite. Dissolve 500 mg of sodium citrate in 10 mL of this solution, and mix.

Buffer Solution—In a 50-mL volumetric flask mix 4.2 g of sodium bicarbonate, 5.0 g of potassium bicarbonate, and 18 mL of water (not all of the solids will dissolve at this stage). To another 18 mL of water add 3.75 g of aminoacetic acid and 1.7 mL of 6 N ammonium hydroxide, mix to dissolve, and transfer this solution to the 50-mL volumetric flask containing the other mixture. Dilute with water to volume, and mix until solution is complete.

Standard Preparation—Transfer about 18 mg of USP Epinephrine Bitartrate RS, accurately weighed, to a 100-mL volumetric flask with the aid of 20 mL of sodium bisulfite solution (1 in 50), dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with sodium bisulfite solution (1 in 500) to volume, and mix. [NOTE—Make the final dilution when the assay is carried out.] The concentration of USP Epinephrine Bitartrate RS in the *Standard Preparation* is about 18 µg per mL.

Assay Preparation—Transfer to a 50-mL volumetric flask an accurately measured volume of the Injection under assay, equivalent to about 500 µg of epinephrine, dilute with sodium bisulfite solution (1 in 500) to volume, if necessary, and mix. [NOTE—The final concentration of sodium bisulfite is in the range of 1 to 3 mg per mL, any bisulfite present in the Injection under assay being taken into consideration.]

Procedure—Into three 50-mL glass-stoppered conical flasks transfer, separately, 20.0-mL aliquots of the *Standard Preparation*, the *Assay Preparation*, and sodium bisulfite solution (1 in 500) to provide the blank. To each flask add 200 µL of *Ferro-citrate Solution* and 2.0 mL of *Buffer Solution*, mix, and allow the solutions to stand for 30 minutes. Determine the absorbances of the solutions in 5-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of epinephrine ($C_9H_{13}NO_3$) in each mL of the Injection taken by the formula:

$$(183.21/333.30)(0.05C/V)(A_U/A_S)$$

in which 183.21 and 333.30 are the molecular weights of epinephrine and epinephrine bitartrate, respectively; C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard Preparation;* and V is the volume, in mL, of Injection taken.

⟨401⟩ FATS AND FIXED OILS

The following definitions and general procedures apply to fats, fixed oils, waxes, resins, balsams, and similar substances.

PREPARATION OF SPECIMEN

If a specimen of oil shows turbidity owing to separated stearin, warm the container in a water bath at 50° until the oil is clear, or if the oil does not become clear on warming, pass it through dry filter paper in a funnel contained in a hot-water jacket. Mix thoroughly, and weigh at one time as many portions as are needed for the various determinations, using preferably a bottle having a pipet dropper, or a weighing buret. Keep the specimen melted, if solid at room temperature, until the desired portions of specimen are withdrawn.

SPECIFIC GRAVITY

Determine the specific gravity of a fat or oil as directed under *Specific Gravity* ⟨841⟩.

MELTING TEMPERATURE

Determine the melting temperature as directed for substances of *Class II* (see *Melting Range or Temperature* ⟨741⟩).

ACID VALUE (FREE FATTY ACIDS)

The acidity of fats and fixed oils in this Pharmacopeia may be expressed as the number of mL of 0.1 N alkali required to neutralize the free acids in 10.0 g of substance. Acidity is frequently expressed as the Acid Value, which is the number of mg of potassium hydroxide required to neutralize the free acids in 1.0 g of the substance.

Procedure—Unless otherwise directed, dissolve about 10.0 g of the substance, accurately weighed, in 50 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 N sodium hydroxide) contained in a flask. If the test specimen does not dissolve in the cold solvent, connect the flask with a suitable condenser and warm slowly, with frequent shaking, until the specimen dissolves. Add 1 mL of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS until the solution remains faintly pink after shaking for 30 seconds. Calculate either the Acid Value or the volume of 0.1 N alkali required to neutralize 10.0 g of specimen (free fatty acids), whichever is appropriate. Calculate the Acid Value by the formula:

$$56.1V \times N/W$$

in which V is the volume, in mL, and N is the normality, respectively, of the sodium hydroxide solution; and W is the weight, in g, of the sample taken.

If the volume of 0.1 N sodium hydroxide VS required for the titration is less than 2 mL, a more dilute titrant may be used, or the sample size may be adjusted accordingly. The results may be expressed in terms of the volume of titrant used or in terms of the equivalent volume of 0.1 N sodium hydroxide.

If the oil has been saturated with carbon dioxide for the purpose of preservation, gently reflux the alcohol-ether solution for 10 minutes before titration. The oil may be freed from carbon dioxide also by exposing it in a shallow dish in a vacuum desiccator for 24 hours before weighing the test specimens.

ESTER VALUE

The Ester Value is the number of mg of potassium hydroxide required to saponify the esters in 1.0 g of the substance. If the *Saponification Value* and the *Acid Value* have been determined, the difference between these two represents the Ester Value.

Procedure—Place 1.5 g to 2 g of the substance in a tared, 250-mL flask, weigh accurately, add 20 mL to 30 mL of neutralized alcohol, and shake. Add 1 mL of phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS until the free acid is neutralized. Add 25.0 mL of 0.5 N alcoholic potassium hydroxide VS, and proceed as directed under *Saponification Value*, beginning with "Heat the flask" and omitting the further addition of phenolphthalein TS. The difference between the volumes, in mL, of 0.5 N

hydrochloric acid consumed in the actual test and in the blank test, multiplied by 28.05 and divided by the weight in g of the specimen taken, is the Ester Value.

HYDROXYL VALUE

The Hydroxyl Value is the number of mg of potassium hydroxide equivalent to the hydroxyl content of 1.0 g of the substance.

Pyridine–Acetic Anhydride Reagent—Just before use, mix 3 volumes of freshly opened or freshly distilled pyridine with 1 volume of freshly opened or freshly distilled acetic anhydride.

Procedure—Transfer a quantity of the substance, determined by reference to the *accompanying table* and accurately weighed, to a glass-stoppered, 250-mL conical flask, and add 5.0 mL of *Pyridine–Acetic Anhydride Reagent*. Transfer 5.0 mL of *Pyridine–Acetic Anhydride Reagent* to a second glass-stoppered, 250-mL conical flask to provide the reagent blank. Fit both flasks with suitable glass-jointed reflux condensers, heat on a steam bath for 1 hour, add 10 mL of water through each condenser, and heat on the steam bath for 10 minutes more. Cool, and to each add 25 mL of butyl alcohol, previously neutralized to phenolphthalein TS with 0.5 N alcoholic potassium hydroxide, by pouring 15 mL through each condenser and, after removing the condensers, washing the sides of both flasks with the remaining 10-mL portions. To each flask add 1 mL of phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS, recording the volume, in mL, consumed by the residual acid in the test solution as T and that consumed by the blank as B. In a 125-mL conical flask, mix about 10 g of the substance, accurately weighed, with 10 mL of freshly distilled pyridine, previously neutralized to phenolphthalein TS, add 1 mL of phenolphthalein TS, and titrate with 0.5 N alcoholic potassium hydroxide VS, recording the volume, in mL, consumed by the free acid in the test specimen as A, or use the Acid Value to obtain A. Calculate the Hydroxyl Value taken by the formula:

$$(56.11N/W)[B + (WA/C) - T]$$

in which W and C are the weights, in g, of the substances taken for the acetylation and for the free acid determination, respectively; N is the exact normality of the alcoholic potassium hydroxide; and 56.11 is the molecular weight of potassium hydroxide.

Hydroxyl Value Range	Weight of Test Specimen, g
0 to 20	10
20 to 50	5
50 to 100	3
100 to 150	2
150 to 200	1.5
200 to 250	1.25
250 to 300	1.0
300 to 350	0.75

IODINE VALUE

The Iodine Value represents the number of g of iodine absorbed, under the prescribed conditions, by 100 g of the substance. Unless otherwise specified in the individual monograph, determine the Iodine Value by *Method I*.

Method I (Hanus Method)

Procedure—Transfer an accurately weighed quantity of sample, as determined from the accompanying table, into a 250-mL iodine flask, dissolve it in 10 mL of chloroform, add 25.0 mL of iodobromide TS, insert the stopper in the vessel securely, and allow it to stand for 30 minutes protected from light, with occasional shaking. Then add, in the order named, 30 mL of potassium iodide TS and 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, shaking thoroughly after each addition of thiosulfate. When the iodine color becomes quite pale, add 3 mL of starch TS, and continue the titration with 0.1 N sodium thiosulfate VS until the blue color is discharged. Perform a blank test at the same time with the same quantities of the same reagents and in the same manner (see *Residual Titrations* ⟨541⟩). Calculate the Iodine Value from the formula:

$$[126.9(V_B - V_S)N]/10W$$

in which 126.9 is the atomic weight of iodine; V_B and V_S are the volumes, in mL, of 0.1 N sodium thiosulfate VS consumed by the blank test and the actual test, respectively; N is the exact normality of the sodium thiosulfate VS; and W is the weight, in g, of the substance taken for the test. [NOTE—If more than half of the iodobromide TS is absorbed by the portion of the substance taken, repeat the determination, using a smaller portion of the substance under examination.]

Sample Weights

Iodine value expected	Weight in g, ±0.001
<5	3.000
5–20	1.000
21–50	0.400
51–100	0.200
101–150	0.130
151–200	0.100

Method II

Potassium Iodide Solution—Dissolve 10.0 g of potassium iodide in water to make 100 mL. Store in light-resistant containers.

Starch Indicator Solution—Mix 1 g of soluble starch with sufficient cold water to make a thin paste. Add, while stirring, to 100 mL of boiling water. Mix, and cool. Use only the clear solution.

Procedure—Melt the sample, if it is not already liquid. [NOTE—The temperature during melting should not exceed the melting point of the sample by more than 10°.] Pass through two pieces of filter paper to remove any solid impurities and the last traces of moisture. The filtration may be performed in an air oven at 100° but should be completed within 5 minutes ± 30 seconds. The sample must be absolutely dry. All glassware must be absolutely clean and completely dry. After filtration, allow the filtered sample to achieve a temperature of 68° to 71 ± 1° before weighing the sample. Once the sample has achieved a temperature of 68° to 71 ± 1°, immediately weigh the sample into a 500-mL iodine flask, using the weights and weighing accuracy noted in the accompanying table. [NOTE—The weight of the substance must be such that there will be an excess of iodochloride TS of 50% to 60% of the amount added, that is, 100% to 150% of the amount absorbed.] Add 15 mL of a fresh mixture of cyclohexane and glacial acetic acid (1 : 1), and swirl to dissolve the sample. Add 25.0 mL of iodochloride TS, insert the stopper securely in the flask, and swirl to mix. Allow it to stand at 25 ± 5°, protected from light, with occasional shaking, for 1.0 or 2.0 hours, depending on the Iodine Value (IV) of the sample: IV less than 150, 1.0 hour; IV equal to or greater than 150, 2.0 hours. Then, within 3 minutes after the indicated reaction time, add, in the order named, 20 mL of *Potassium Iodide Solution* and 150 mL of recently boiled and cooled water, and mix. Within 30 minutes, titrate the liberated iodine with 0.1 N sodium thiosulfate VS, while stirring by mechanical means after each addition of thiosulfate. When the yellow iodine color has almost disappeared, add 1 to 2 mL of *Starch Indicator Solution*, and continue the titration with 0.1 N sodium thiosulfate VS until the blue color is discharged. Perform a blank test at the same time with the same quantities of the same reagents and in the same manner (see *Residual Titrations* ⟨541⟩). The difference between the volumes, in mL, of 0.1 N sodium thiosulfate consumed by the blank test and the actual test, multiplied by 1.269 and divided by the weight, in g, of the sample taken, is the Iodine Value.

PEROXIDE VALUE

The Peroxide Value is the number that expresses, in milliequivalents of active oxygen, the quantity of peroxide contained in 1000 g of the substance. [NOTE—This test must be performed promptly after sampling to avoid oxidation of the test specimen.]

Procedure—Unless otherwise directed, place about 5 g of the substance, accurately weighed, in a 250-mL conical flask fitted with a ground-glass stopper. Add 30 mL of a mixture of glacial acetic acid and chloroform (3:2), shake to dissolve, and add 0.5 mL of saturated potassium iodide solution. Shake for exactly 1 minute, and add 30 mL of water. Titrate with 0.01 N sodium thiosulfate VS, adding the titrant slowly with continuous shaking, until the yellow color is almost discharged. Add 5 mL of starch TS, and continue the titration, shaking vigorously, until the blue color is discharged. Perform a blank determination under the same conditions. [NOTE—The volume of titrant used in the blank determination must not exceed 0.1 mL.] The difference between the volumes, in mL, of 0.01 N sodium thiosulfate consumed in the actual test and in the blank test, multiplied by 10 and divided by the weight, in g, of the specimen taken, is the Peroxide Value.

SAPONIFICATION VALUE

The Saponification Value is the number of mg of potassium hydroxide required to neutralize the free acids and saponify the esters contained in 1.0 g of the substance.

Procedure—Place 1.5 g to 2 g of the substance in a tared, 250-mL flask, weigh accurately, and add to it 25.0 mL of 0.5 N alcoholic potassium hydroxide. Heat the flask on a steam bath, under a suitable condenser to maintain reflux for 30 minutes, frequently rotating the contents. Then add 1 mL of phenolphthalein TS, and titrate the excess potassium hydroxide with 0.5 N hydrochloric acid VS. Perform a blank determination under the same conditions (see *Residual Titrations* under *Titrimetry* ⟨541⟩). The titration also can be carried out potentiometrically. The difference between the volumes, in mL, of 0.5 N hydrochloric acid consumed in the actual test and in the blank test, multiplied by 56.1 and the exact normality of the 0.5 N hydrochloric acid VS, and divided by the weight in g of specimen taken, is the Saponification Value.

If the oil has been saturated with carbon dioxide for the purpose of preservation, expose it in a shallow dish in a vacuum desiccator for 24 hours before weighing the test specimens.

UNSAPONIFIABLE MATTER

The term "Unsaponifiable Matter" in oils or fats, refers to those substances that are not saponifiable by alkali hydroxides but are soluble in the ordinary fat solvents, and to products of saponification that are soluble in such solvents.

Procedure—Transfer about 5.0 g of the oil or fat, accurately weighed, to a 250-mL conical flask, add 50 mL of an alcoholic potassium hydroxide solution prepared by dissolving 12 g of potassium hydroxide in 10 mL of water and diluting this solution with alcohol to 100 mL, and heat the flask on a steam bath under a suitable condenser to maintain reflux for 1 hour, swirling frequently. Cool to a temperature below 25°, and transfer the contents of the flask to a separator having a polytetrafluoroethylene stopcock, rinsing the flask with two 50-mL portions of water that are added to the separator (do not use grease on stopcock). Extract with three 100-mL portions of ether, combining the ether extracts in another separator containing 40 mL of water. Gently rotate or shake the separator for a few minutes. [NOTE—Violent agitation may result in the formation of a difficult-to-separate emulsion.] Allow the mixture to separate, and discard the lower aqueous phase. Wash the ether extract with two additional 40-mL portions of water, and discard the lower aqueous phase. Wash the ether extract successively with a 40-mL portion of potassium hydroxide solution (3 in 100) and a 40-mL portion of water. Repeat this potassium hydroxide solution-water wash sequence three times. Wash the ether extract with 40-mL portions of water until the last washing is not reddened by the addition of 2 drops of phenolphthalein TS. Transfer the ether extract to a tared flask, and rinse the separator with 10 mL of ether, adding the rinsings to the flask. Evaporate the ether on a steam bath, and add 6 mL of acetone to the residue. Remove the acetone in a current of air, and dry the residue at 105° until successive weighings differ by not more than 1 mg. Calculate the percentage of unsaponifiable matter in the portion of oil or fat taken by the formula:

$$100(W_R / W_S)$$

in which W_R is the weight, in g, of the residue; and W_S is the weight, in g, of the oil or fat taken for the test.

Dissolve the residue in 20 mL of alcohol, previously neutralized to the phenolphthalein endpoint, add phenolphthalein TS, and titrate with 0.1 N alcoholic sodium hydroxide VS to the first appearance of a faint pink color that persists for not less than 30 seconds. If the volume of 0.1 N alcoholic sodium hydroxide required is greater than 0.2 mL, the separation of the layers was incomplete; the residue weighed cannot be considered as "unsaponifiable matter," and the test must be repeated.

SOLIDIFICATION TEMPERATURE OF FATTY ACIDS

Preparation of the Fatty Acids—Heat 75 mL of glycerin–potassium hydroxide solution (made by dissolving 25 g of potassium hydroxide in 100 mL of glycerin) in an 800-mL beaker to 150°, and add 50 mL of the clarified fat, melted if necessary. Heat the mixture for 15 minutes with frequent stirring, but do not allow the temperature to rise above 150°. Saponification is complete when the mixture is homogeneous, with no particles clinging to the beaker at the meniscus. Pour the contents of the beaker into 500 mL of nearly boiling water in an 800-mL beaker or casserole, add slowly 50 mL of dilute sulfuric acid (made by adding water and sulfuric acid (3:1)), and heat the solution, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Wash the acids with boiling water until free from sulfuric acid, collect them in a small beaker, place on a steam bath until the water has settled and the fatty acids are clear, filter into a dry beaker while hot, and dry at 105° for 20 minutes. Place the warm fatty acids in a suitable container, and cool in an ice bath until they congeal.

Test for Complete Saponification—Place 3 mL of the dry acids in a test tube, and add 15 mL of alcohol. Heat the solution to boiling, and add an equal volume of 6 N ammonium hydroxide. A clear solution results.

Procedure—Using an apparatus similar to the "Congealing Temperature Apparatus" specified therein, proceed as directed for *Procedure* under *Congealing Temperature* ⟨651⟩, reading "solidification temperature" for "congealing point" (the terms are synonymous). The average of not less than four consecutive readings of the highest point to which the temperature rises is the solidification temperature of the fatty acids.

FATTY ACID COMPOSITION

Standard Solution—Prepare an ester mixture of known composition containing the esters required in the individual monograph. This *Standard Solution* may contain other components. [NOTE—Ester mixtures are available commercially from Nu-Chek-Prep, Inc., P.O. Box 295, Elysian, MN 56028. Typical Nu-Chek-Prep ester mixtures useful in this test include Nu-Chek 17A and Nu-Chek 19A.] Nu-Chek mixture 17A has the following composition:

Percentage	Fatty Acid Ester	Carbon-chain Length	No. of Double Bonds
1.0	methyl myristate	14	0
4.0	methyl palmitate	16	0
3.0	methyl stearate	18	0
3.0	methyl arachidate	20	0
3.0	methyl behenate	22	0
3.0	methyl lignocerate	24	0
45.0	methyl oleate	18	1
15.0	methyl linoleate	18	2

Percentage	Fatty Acid Ester	Carbon-chain Length	No. of Double Bonds
3.0	methyl linolenate	18	3
20.0	methyl erucate	22	1

Nu-Chek mixture 19A has the following composition:

Percentage	Fatty Acid Ester	Carbon-chain Length	No. of Double Bonds
7.0	methy caprylate	8	0
5.0	methyl caprate	10	0
48.0	methyl laurate	12	0
15.0	methyl myristate	14	0
7.0	methyl palmitate	16	0
3.0	methyl stearate	18	0
12.0	methyl oleate	18	1
3.0	methyl linoleate	18	2

Test Solution—[NOTE—If fatty acids containing more than 2 double bonds are present in the test specimen, remove air from the flask by purging it with nitrogen for a few minutes.] Transfer about 100 mg of the test specimen to a 50-mL conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar. Add 4 mL of 0.5 N methanolic sodium hydroxide solution, and reflux until fat globules disappear (usually 5 to 10 minutes). Add 5 mL of a solution prepared by dissolving 14 g of boron trifluoride in methanol to make 100 mL, swirl to mix, and reflux for 2 minutes. Add 4 mL of chromatographic *n*-heptane through the condenser, and reflux for 1 minute. Cool, remove the condenser, add about 15 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Pass the *n*-heptane layer through 0.1 g of anhydrous sodium sulfate (previously washed with chromatographic *n*-heptane) into a suitable flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with chromatographic *n*-heptane to volume, and mix.

System Suitability Solution—Transfer about 20 mg each of stearic acid, palmitic acid and oleic acid to a 25-mL conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar, and proceed as directed for *Test Solution,* beginning with "Add 5.0 mL of a solution prepared by dissolving."

Chromatographic System (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, maintained at a temperature of about 260°, a splitless injection system, and a 0.53-mm × 30-m fused-silica capillary column bonded with a 1.0-µm layer of phase G16. The chromatograph is programmed to maintain the column temperature at 70° for about 2 minutes after injection, then to increase the temperature at the rate of 5° per minute to 240°, and finally to maintain this temperature for 5 minutes. The injection port temperature is maintained at about 220°. The carrier gas is helium with a linear velocity of about 50 cm per second.

Chromatograph the *System Suitability Solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.87 for methyl palmitate, 0.99 for methyl stearate, and 1.0 for methyl oleate; the resolution, *R,* between methyl stearate and methyl oleate is not less than 1.5; and the relative standard deviation of the peak area responses for the palmitate and stearate peaks for replicate injections is not more than 6.0%. The relative standard deviation of the peak area response ratio of the palmitate to stearate peaks from these replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 1 µL) of the *Standard Solution* and the *Test Solution* into the chromatograph, record the chromatograms, identify the fatty acid ester peaks in the chromatogram of the *Test Solution* by comparing the retention times of these peaks with those obtained in the chromatogram of the *Standard Solution,* and measure the peak areas for all of the fatty acid ester peaks in the chromatogram obtained from the *Test Solution*. Calculate the percentage of each fatty acid component in the test specimen by the formula:

$$100(A/B)$$

in which A is the area of the peak response obtained for each individual fatty acid ester component; and B is the sum of the peak areas of all of the peaks, excluding the solvent peak, in the chromatogram obtained from the *Test Solution.*

WATER AND SEDIMENT IN FIXED OILS

Apparatus—The preferred centrifuge has a diameter of swing (d = distance from tip to tip of whirling tubes) of 38 to 43 cm and is operated at a speed of about 1500 rpm. If a centrifuge of different dimensions is used, calculate the desired rate of revolution by the formula:

$$\text{rpm} = 1500\sqrt{40.6/d}.$$

The centrifuge tubes are pear-shaped, and are shaped to accept closures. The total capacity of each tube is about 125 mL. The graduations are clear and distinct, reading upward from the bottom of the tube according to the scale shown in the *accompanying table.*

Volume (mL)	Scale Division (mL)
0 to 3	0.1
3 to 5	0.5
5 to 10	1.0
10 to 25	5.0
25 to 50	25.0
50 to 100	50.0

Procedure—Place 50.0 mL of benzene in each of two centrifuge tubes, and to each tube add 50.0 mL of the oil, warmed if necessary to re-incorporate separated stearin, and mixed thoroughly at 25°. Insert the stopper tightly into the tubes, and shake them vigorously until the contents are mixed thoroughly, then immerse the tubes in a water bath at 50° for 10 minutes. Centrifuge for 10 minutes. Read the combined volume of water and sediment at the bottom of each tube. Centrifuge repeatedly for 10-minute periods until the combined volume of water and sediment remains constant for 3 consecutive readings. The sum of the volumes of combined water and sediment in the two tubes represents the percentage, by volume, of water and sediment in the oil.

ANISIDINE VALUE

The anisidine value is defined as 100 times the optical density measured in a 1-cm cell of a solution containing 1 g of the substance to be examined in 100 mL of a mixture of solvents and reagents according to the method described below. [NOTE—Carry out the operations as rapidly as possible, avoiding exposure to actinic light.]

Test Solution A—Dissolve 0.500 g of the substance to be examined in isooctane, and dilute with the same solvent to 25.0 mL.

Test Solution B—To 5.0 mL of *Test Solution A* add 1.0 mL of a 2.5 g per L solution of *p*-anisidine in glacial acetic acid, shake, and store protected from light.

Standard Solution—To 5.0 mL of isooctane add 1.0 mL of a 2.5 g per L solution of *p*-anisidine in glacial acetic acid, shake, and store protected from light.

Procedure—Measure the absorbance of *Test Solution A* at 350 nm using isooctane as the blank. Measure the absorbance of *Test Solution B* at 350 nm exactly 10 minutes after its preparation, using the *Standard Solution* as the compensation liquid. Calculate the Anisidine Value from the expression:

$$\frac{25(1.2A_s - A_b)}{m},$$

in which A_s is the absorbance of *Test Solution B* at 350 nm; A_b is the absorbance of *Test Solution A* at 350 nm; and m is the weight, in g, of the substance to be examined in *Test Solution A*.

TOTAL OXIDATION VALUE (TOTOX)

Total Oxidation Value is defined by the formula:

$$2PV + AV$$

in which *PV* is the *Peroxide Value*, and *AV* is the *Anisidine Value*.

⟨411⟩ FOLIC ACID ASSAY

The following procedure is provided for the estimation of folic acid as an ingredient of Pharmacopeial preparations containing other active constituents.

USP Reference Standards ⟨11⟩—*USP Folic Acid RS*.

Mobile Phase—Place 2.0 g of monobasic potassium phosphate in a 1-liter volumetric flask, and dissolve in about 650 mL of water. Add 12.0 mL of a 1 in 4 solution of tetrabutylammonium hydroxide in methanol, 7.0 mL of 3 N phosphoric acid, and 240 mL of methanol. Cool to room temperature, adjust with either 3 N phosphoric acid or 6 N ammonium hydroxide to a pH of 7.0, dilute with water to volume, and mix. Pass through a 0.45-µm filter, and recheck the pH before use. [NOTE—The methanol-to-water ratio may be varied by up to 3 percent and the pH may be increased up to 7.15 to achieve better separation.]

Diluting Solvent—Prepare as directed under *Mobile Phase*. Adjust to a pH of 7.0, and bubble nitrogen through the solution for 30 minutes before use.

Internal Standard Solution—Dissolve about 25 mg of methylparaben in 2.0 mL of methanol, dilute with *Diluting Solvent* to 50 mL, and mix.

Standard Folic Acid Solution—Transfer about 12 mg of USP Folic Acid RS, accurately weighed, to a low-actinic, 50-mL volumetric flask, dissolve in 2 mL of ammonium hydroxide, dilute with *Diluting Solvent* to volume, and mix.

Standard Preparation—Transfer 2.0 mL of *Standard Folic Acid Solution* to a low-actinic, 25-mL volumetric flask, add 2.0 mL of *Internal Standard Solution*, add *Diluting Solvent* to volume, and mix.

Assay Preparation—Transfer an accurately weighed or measured portion of the preparation to be assayed, containing about 1 mg of folic acid, to a low-actinic, 50-mL volumetric flask, add 4.0 mL of *Internal Standard Solution*, add *Diluting Solvent* to volume, and mix.

Chromatographic System (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 15-cm × 3.9-mm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard Preparation*, and record the peak responses as directed for *Procedure*: there is baseline separation of folic acid and methylparaben.

Procedure—Separately inject equal volumes (about 10 µL) of *Standard Preparation* and *Assay Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for folic acid and 1.0 for methylparaben. Calculate the quantity, in µg, of $C_{19}H_{19}N_7O_6$ in the portion of the preparation taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in µg per mL, of USP Folic Acid RS in the *Standard Preparation*; and R_U and R_S are the ratios of the response of the folic acid peak to that of the methylparaben peak obtained from the *Assay Preparation* and the *Standard Preparation*, respectively.

⟨425⟩ IODOMETRIC ASSAY—ANTIBIOTICS

The following method is provided for the assay of most of the Pharmacopeial penicillin antibiotic drugs and their dosage forms, for which iodometric titration is particularly suitable.

Standard Preparation—Dissolve in the solvent specified in the table of *Solvents and Final Concentrations* a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried under the conditions specified in the individual monograph and accurately weighed, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about that specified in the table. Pipet 2.0 mL of this solution into each of two 125-mL glass-stoppered conical flasks.

Solvents and Final Concentrations

Antibiotic	Solvent*	Final Concentration
Amoxicillin	Water	1.0 mg per mL
Ampicillin	Water	1.25 mg per mL
Ampicillin Sodium	*Buffer No. 1*	1.25 mg per mL
Cloxacillin Sodium	Water	1.25 mg per mL
Cyclacillin	Water	1.0 mg per mL
Dicloxacillin Sodium	*Buffer No. 1*	1.25 mg per mL
Methicillin Sodium	*Buffer No. 1*	1.25 mg per mL
Nafcillin Sodium	*Buffer No. 1*	1.25 mg per mL
Oxacillin Sodium	*Buffer No. 1*	1.25 mg per mL
Penicillin G Potassium	*Buffer No. 1*	2,000 units per mL
Penicillin G Sodium	*Buffer No. 1*	2,000 units per mL
Penicillin V Potassium	*Buffer No. 1*	2,000 units per mL
Phenethicillin Potassium	*Buffer No. 1*	2,000 units per mL

* Unless otherwise noted, the *Buffers* are the potassium phosphate buffers defined in the section *Media and Diluents* under *Antibiotics—Microbial Assays* ⟨81⟩, except that sterilization is not required before use.

Assay Preparation—Unless otherwise specified in the individual monograph, dissolve in the solvent specified in the table of *Solvents and Final Concentrations* a suitable quantity, accurately weighed, of the specimen under test, and dilute quantitatively with the same solvent to obtain a solution having a known final concentration of about that specified in the table. Pipet 2 mL of this solution into each of two 125-mL glass-stoppered conical flasks.

Procedure—

Inactivation and *Titration*—To 2.0 mL of the *Standard Preparation* and of the *Assay Preparation*, in respective flasks, add 2.0 mL of 1.0 N sodium hydroxide, mix by swirling, and allow to stand for 15 minutes. To each flask add 2.0 mL of 1.2 N hydrochloric acid, add 10.0 mL of 0.01 N iodine VS, immediately insert the stopper, and allow to stand for 15 minutes. Titrate with 0.01 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, and continue the titration to the discharge of the blue color.